**Citation:**

Greene CM, Zern TL, Wood RJ, Shrestha S, Aggarwal D, Sharman MJ, Volek JS, Fernandez ML. Maintenance of the LDL cholesterol:HDL cholesterol ratio in an elderly population given a dietary cholesterol challenge. *J Nutr.* 2005 Dec;135(12):2793-8.

**PubMed ID:** 16317122

**Study Design:**

Randomized Crossover Trial

**Class:**

A - Click here for explanation of classification scheme.

**Research Design and Implementation Rating:**

NEUTRAL: See Research Design and Implementation Criteria Checklist below.

**Research Purpose:**

- To evaluate the effects of a cholesterol challenge on plasma cholesterol, LDL size, and LDL susceptibility to oxidation in the elderly.
- The study hypothesis was that the consumption of the equivalent of 3 eggs per day would not increase the risk for CHD in this population.

**Inclusion Criteria:**

- Men who were greater than 60 years old.
- Women who were postmenopausal for at least one year.

**Exclusion Criteria:**

- Those who were taking prescription medication for elevated lipids
- Those with an egg allergy
- Those with a history of heart disease, diabetes, heart disease, or kidney problems
- Total cholesterol concentration at baseline of > 6.2 mmol/L (240 mg/dL) or total triglyceride levels > 3.4 mmol/L (300 mg/dL).

**Description of Study Protocol:**

**Recruitment** :Participants were recruited through local newspapers and brochure distribution within a university community.

**Design:** Randomized crossover trial

- Subjects were assigned to an EGG (equivalent of 3 eggs/day containing approximately 640 mg of dietary cholesterol) or SUB (equal volume of cholesterol-free, fat-free product almost

identical in color and consistency to the egg product containing 0 mg of dietary cholesterol) group for 30 days, followed by a 3 week washout period.
- The subjects were then crossed over to the alternate intervention and continued for 30 days.
- Subjects were advised to avoid additional egg consumption outside of that provided by the study and to maintain their regular diets.
- Two, 7-day dietary records were collected that included 2 weekend days and 5 nonconsecutive weekdays.
- Two fasting blood draws were scheduled were scheduled at the end of each intervention period, on nonconsecutive days, 2 days apart, for each subject.

**Blinding used (if applicable):** Neither subjects nor investigators were blinded.

**Intervention (if applicable)**: An egg product which was the equivalent of 3 eggs/day (approximately 640 mg of dietary cholesterol).

**Statistical Analysis:**

Repeated measures ANOVA was used to analyze the effects of dietary cholesterol on plasma lipids, lipoprotein metabolism, and LDL particle characteristics.

**Data Collection Summary:**

**Timing of Measurements:**

- The study included two 30-day intervention periods and a 3-week washout period.
- Two fasting blood draws were scheduled were scheduled at the end of each intervention period, on nonconsecutive days, 2 days apart, for each subject.

**Dependent Variables**

- Total cholesterol as measured by enzymatic methods.
- HDL-cholesterol as measured by in a supernatant after precipitation of the apo-B containing lipoproteins.
- LDL-cholesterol as calculated by the Friedewald equation.
- TG as measured using Roche diagnostic kits.
- Apo-B as measured by Sigma Chemical kits.
- LDL particle size as measured by the Lipoprint LDL system.
- LDL oxidation as measured by the Lowrey method.
- Plasma lecithin cholesteryl acyl transferase (LCAT) as measure by analysis of the decrease in plasma free cholesterol.
- Cholesteryl ester transfer protein (CETP) as measured by calculations.

**Independent Variables**

- Subjects were assigned to an EGG (equivalent of 3 eggs/day containing approximately 640 mg of dietary cholesterol) or SUB (equal volume of cholesterol-free, fat-free product almost identical in color and consistency to the egg product containing 0 mg of dietary cholesterol) group for 30 days, followed by a 3 week washout period.
- The subjects were then crossed over to the alternate intervention and continued for 30 days.
- Subjects were advised to avoid additional egg consumption outside of that provided by the study and to maintain their regular diets.
- Two, 7-day dietary records were collected that included 2 weekend days and 5

nonconsecutive weekdays.

**Control Variables**

**Description of Actual Data Sample:**

**Initial N**: 42 volunteers were accepted into the study, 13 men and 29 women.

**Attrition (final N):** All those who entered the study completed it; there was no attrition.

**Age**: Men were greater than 60 years old. Age of female participants was not specified but they were all at least one year post-menopause.

**Ethnicity**: Not specified

**Other relevant demographics**: No information was provided

**Anthropometrics:**

- At baseline, men had a BMI of 26.1 ± 2.9 kg/m$^2$ and women had a BMI of 27.8 ± 5.9 kg/m$^2$.
- Men had a waist circumference of 96.6 ± 9.9 cm and women had a waist circumference of 87.3 ± 14.6 cm.
- Waist circumference was greater in men ($P < 0.05$) than in women, and HDL-C concentrations were greater in women ($P < 0.05$) than in men

**Location**: Storrs, CT

**Summary of Results:**

**Key Findings:**

- In both men and women, TC, LDL-C ($P < 0.05$), and HDL-C ($P < 0.001$) increased as a result of the egg supplementation
- The LDL:HDL ratio and plasma TG did not differ between the EGG and SUB periods.
- The apo-B concentration was not affected by egg consumption
- The LDL particle size did increase during the EGG period compared with the SUB period
- There were no changes in the parameters of LDL oxidation, conjugated dienes and lag time for men and women during the EGG period vs the SUB periods.

**Plasma TC, LDL-C, HDL-C and TG of men and women after the EGG or SUB periods**

| Subjects | TC | LDL-C | HDL-C | TG | LDL-C:HDL-C |
|---|---|---|---|---|---|
| Men (n = 13) | | | | | |
| EGG | 176.8±30.0 | 107.4± 27.8 | 51.9±12.9 | 87.2±34.9 | 2.2±1.1 |
| SUB | 170.8±25.9 | 105.2±32.4 | 47.2±12.9 | 96.5±49.1 | 2.4±1.1 |

| | | | | | |
|---|---|---|---|---|---|
| Women(n=29) | 195.5±38.8 | 115.3±40.7 | 59.2±14.7 | 104.6±54.7 | 2.2±1.1 |
| EGG | 183.7±34.0 | 105.2±32.4 | 57.7±12.0 | 104.4±59.3 | 2.0±1.1 |
| SUB | | | | | |
| Repeated -measures ANOVA, p-values | <0.05 | <0.05 | <0.001 | NS | NS |
| | NS | NS | NS | NS | <0.05 |
| Diet effect | NS | NS | NS | NS | NS |
| Gender effect | | | | | |
| Interaction | | | | | |

Values are means ±SD, NS=not significant, p≥0.05

**Plasma cholesterol in the LDL subfractions, LDL diameter, and apo B Concentrations**

| Subjects | Plasma apo B | LDL-1 | LDL-2 | LDL-3 | LDL Diameter |
|---|---|---|---|---|---|
| Men (n = 13) | 796±147 | 60.2±20.7 | 18.9±8.0 | 13.0±8.7 | 26.2±1.2 |
| EGG | 847±183 | 61.5±18.5 | 15.6±8.2 | 12.9±9.9 | 25.7±1.2 |
| SUB | | | | | |
| Women(n=29) | 879±208 | 60.2±20.8 | 18.3±10.2 | 12.9±13.1 | 26.1±1.4 |
| EGG | 799±177 | 60.9±26.5 | 17.8±7.8 | 15.0±18.0 | 25.8±1.8 |
| SUB | | | | | |
| Repeated -measures ANOVA, p-values | NS | NS | NS | NS | <0.05 |
| | NS | NS | NS | NS | NS |
| Diet effect | NS | NS | NS | NS | NS |
| Gender effect | | | | | |
| Interaction | | | | | |

Values are means ±SD, NS=not significant, p≥0.05

**Other Findings**

- The men and women had a significant increase in their total fat, SFA, MUFA, and cholesterol intakes during the EGG period.

**Author Conclusion:**

In summary, these results show that postmenopausal women and men > 60 years old with healthy lipoprotein profiles may consume eggs as part of their regular diet. For those subjects who experienced an increase in LDL-C in response to the dietary cholesterol challenge, this was countered by an increase in HDL-C and an increase in the size of the LDL particle (antiatherogenic). In addition, the susceptibility of LDL to oxidation is not enhanced by egg intake.

***Reviewer Comments:***

- *The n in this study was relatively small, and there were significant differences between the men and women*
- *Sponsored by the egg industry*

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***Research Design and Implementation Criteria Checklist: Primary Research***

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|---|---|---|
| **Relevance Questions** | | |
| 1. | Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies) | Yes |
| 2. | Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about? | Yes |
| 3. | Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice? | Yes |
| 4. | Is the intervention or procedure feasible? (NA for some epidemiological studies) | Yes |
| **Validity Questions** | | |
| **1.** | **Was the research question clearly stated?** | Yes |
| 1.1. | Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified? | Yes |
| 1.2. | Was (were) the outcome(s) [dependent variable(s)] clearly indicated? | Yes |
| 1.3. | Were the target population and setting specified? | Yes |
| **2.** | **Was the selection of study subjects/patients free from bias?** | No |
| 2.1. | Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study? | Yes |
| 2.2. | Were criteria applied equally to all study groups? | Yes |

| | | |
|---|---|---|
| 2.3. | Were health, demographics, and other characteristics of subjects described? | Yes |
| 2.4. | Were the subjects/patients a representative sample of the relevant population? | No |
| **3.** | **Were study groups comparable?** | Yes |
| 3.1. | Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT) | Yes |
| 3.2. | Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline? | Yes |
| 3.3. | Were concurrent controls used? (Concurrent preferred over historical controls.) | Yes |
| 3.4. | If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis? | N/A |
| 3.5. | If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.) | N/A |
| 3.6. | If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")? | N/A |
| **4.** | **Was method of handling withdrawals described?** | Yes |
| 4.1. | Were follow-up methods described and the same for all groups? | Yes |
| 4.2. | Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.) | Yes |
| 4.3. | Were all enrolled subjects/patients (in the original sample) accounted for? | Yes |
| 4.4. | Were reasons for withdrawals similar across groups? | N/A |
| 4.5. | If diagnostic test, was decision to perform reference test not dependent on results of test under study? | N/A |
| **5.** | **Was blinding used to prevent introduction of bias?** | Yes |
| 5.1. | In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate? | N/A |
| 5.2. | Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.) | Yes |

| | | |
|---|---|---|
| 5.3. | In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded? | N/A |
| 5.4. | In case control study, was case definition explicit and case ascertainment not influenced by exposure status? | N/A |
| 5.5. | In diagnostic study, were test results blinded to patient history and other test results? | N/A |
| **6.** | **Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?** | Yes |
| 6.1. | In RCT or other intervention trial, were protocols described for all regimens studied? | Yes |
| 6.2. | In observational study, were interventions, study settings, and clinicians/provider described? | N/A |
| 6.3. | Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect? | Yes |
| 6.4. | Was the amount of exposure and, if relevant, subject/patient compliance measured? | Yes |
| 6.5. | Were co-interventions (e.g., ancillary treatments, other therapies) described? | N/A |
| 6.6. | Were extra or unplanned treatments described? | N/A |
| 6.7. | Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups? | Yes |
| 6.8. | In diagnostic study, were details of test administration and replication sufficient? | N/A |
| **7.** | **Were outcomes clearly defined and the measurements valid and reliable?** | Yes |
| 7.1. | Were primary and secondary endpoints described and relevant to the question? | Yes |
| 7.2. | Were nutrition measures appropriate to question and outcomes of concern? | Yes |
| 7.3. | Was the period of follow-up long enough for important outcome(s) to occur? | Yes |
| 7.4. | Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures? | Yes |
| 7.5. | Was the measurement of effect at an appropriate level of precision? | Yes |
| 7.6. | Were other factors accounted for (measured) that could affect outcomes? | No |
| 7.7. | Were the measurements conducted consistently across groups? | Yes |
| **8.** | **Was the statistical analysis appropriate for the study design and type of outcome indicators?** | Yes |

| | | |
|---|---|---|
| 8.1. | Were statistical analyses adequately described and the results reported appropriately? | Yes |
| 8.2. | Were correct statistical tests used and assumptions of test not violated? | Yes |
| 8.3. | Were statistics reported with levels of significance and/or confidence intervals? | Yes |
| 8.4. | Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)? | N/A |
| 8.5. | Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)? | ??? |
| 8.6. | Was clinical significance as well as statistical significance reported? | Yes |
| 8.7. | If negative findings, was a power calculation reported to address type 2 error? | N/A |
| **9.** | **Are conclusions supported by results with biases and limitations taken into consideration?** | ??? |
| 9.1. | Is there a discussion of findings? | Yes |
| 9.2. | Are biases and study limitations identified and discussed? | No |
| **10.** | **Is bias due to study's funding or sponsorship unlikely?** | No |
| 10.1. | Were sources of funding and investigators' affiliations described? | Yes |
| 10.2. | Was the study free from apparent conflict of interest? | No |